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# sRANKL ELISA Kit

*For the in vitro determination of sRANKL in serum and  
cell culture supernatant*

Gültig ab/valid from 31.10.2005

Artikelnummer/Catalogue No.: IMM-KB 1014

Packungsgröße/Package size: 96 Tests/96 determinations

Lagerung/Storage: 2 - 8 °C



## 1. INTENDED USE

The Immundiagnostik assay is a sandwich ELISA intended for the quantitative determination of sRANKL in serum and cell culture supernatant. For *in vitro* diagnostic use only.

## 2. SUMMARY AND EXPLANATION OF THE TEST

RANKL, receptor activator of nuclear factor (NF)- $\kappa$ B ligand (also: osteoprotegerin ligand, OPGL), its cellular receptor, receptor activator of NF- $\kappa$ B (RANK), and the decoy receptor, osteoprotegerin (OPG) have been identified as the key molecular regulation system for bone remodelling. RANKL, a member of the tumor necrosis factor (TNF) family, is the main stimulatory factor for the formation of mature osteoclasts and is essential for their survival. Therefore, an increase in RANKL expression leads to bone resorption and bone loss. RANKL is produced by osteoblastic lineage cells and activated T lymphocytes. It activates its specific receptor RANK which is located on osteoclasts and dendritic cells.

The effects of RANKL are counteracted by OPG which is secreted by various tissues and acts as an endogenous soluble receptor antagonist.

Imbalances of the RANKL/OPG system have been related to the pathogenesis of Paget's disease, benign and malignant bone tumors, postmenopausal osteoporosis, rheumatoid arthritis, bone metastases and hypercalcemia. It was shown in several studies that in animal models restoring of the RANKL/OPG balance (e.g. by administering OPG) reduces the severity of these disorders.

It has been shown that RANKL is produced as a membrane-bound protein on murine osteoblasts/stromal cells, and cleaved into a soluble form by a metalloprotease. Stimulators of the osteoclastogenesis such as IL-1 $\beta$ , IL-6, IL-11, IL-17, and TNF- $\alpha$ , increase the expression of RANKL and decrease OPG expression in osteoblasts/stromal cells. Cytokines inhibiting the osteoclastogenesis such as IL-13, INF- $\gamma$ , and TGF- $\beta$ 1, suppress the expression of RANKL and stimulated OPG expression.

## Molecular structure

sRANKL is a part of the TNF superfamily with high similarity to other members of that protein species. (SwissProt Nr. O14788). Two isoforms are produced by alternate splicing, a type II membrane protein (ISOFORM 1, 317 AA, MW 35.5 kD), and a secreted molecule (ISOFORM 2, 244 AA MW 27.7kD), lacking the cytoplasmic and transmembrane domain. Although both forms are bioactive, the membrane bound protein seems to be the homeostatic form, while the production of soluble RANKL signals pathological conditions.

## Indications

- Postmenopausal and senile osteoporosis
- Diseases with locally increased bone resorption activity
- Paget´s disease
- Periodontal disease
- Inflammatory diseases
- Immunological disorders
- Arthritis
- Oncology

## 3. PRINCIPLE OF THE TEST

The sRANKL ELISA test kit is an enzyme immunoassay designed to determine soluble, uncomplexed human RANKL directly in biological fluids like serum and cell culture supernatants.

In a first step, sample and the biotinylated anti-sRANKL detection antibody are pipetted into the wells. Human sRANKL, if present in the sample, binds to the precoated recombinant osteoprotegerin (OPG) and forms a sandwich with the detection antibody.

After a washing step, which removes all non-specific bound material, streptavidin-HRP conjugate is added into the wells. After removal of unbound conjugate by washing, tetramethylbenzidine (TMB) is added to the wells as substrate. sRANKL is quantified by an enzyme catalyzed colour change measured on a standard ELISA reader. The developed colour intensity is directly proportional to the amount of sRANKL present in the sample.

#### 4. MATERIAL SUPPLIED

Catalogue no.	Kit Components	Quantity
KB1014MTP	Pre-coated microtiter plate, 12 x 8 strips	96
KB1014WP	ELISA washing buffer concentrate (20x)	50 ml
KB1014ST	Calibrators, lyophilized (0; 0.38; 0.75; 1.5; 3.0; 6.0 pmol/l)	6 x 1 vial
KB1014ST	Stock solution, lyophilized (20 pmol/l)	1 vial
KB1014KO	Control, lyophilized (See data sheet for range)	1 vial
KB1014A2	2 <sup>nd</sup> antibody (anti-OPG, biotinylated), ready-to-use	11 ml
KB1014K	Conjugate, (streptavidin-peroxidase labeled), ready-to-use	22 ml
KB10114MB	TMB substrate (Tetramethylbenzidine), ready-to-use	22 ml
KB1014AC	ELISA stop solution, ready-to-use	1 x 7 ml

#### 5. MATERIAL REQUIRED BUT NOT SUPPLIED

- Distilled or deionized water
- Precision pipettes calibrated to deliver 50 -1000 µl and disposable tips.
- Multichannel or Multipipette
- Vortex mixer
- Conventional glass or plastic tubes
- 1,5 ml reaction vials (Eppendorf)
- ELISA reader equipped with 450 nm filter
- Graph paper or software for calculation of results

## 6. PREPARATION AND STORAGE OF REAGENTS

Store samples at -20°C if not assayed on the same day. Lipaemic or haemolysed samples may give erroneous results. Samples should be mixed well before assaying. We recommend duplicates for all values.

- Dilute washing buffer concentrate to 1000 ml (50 ml concentrate + 950 ml distilled water) and mix well. Crystals in the buffer concentrate will dissolve at room temperature. Buffer is stable at 2-8° C until expiry date stated on the label.
- Mark positions for blank, standards, control, and samples on the protocol sheet supplied.
- Take as many microtiter strips as needed from kit. Store unused strips covered at 2-8° C. Strips are stable until the expiry date stated on the label.
- The **calibrators** and **controls** must be reconstituted with **700 µl** aqua dest. Allow the vial to stand for 10 minutes and then mix thoroughly by gentle inversion to insure complete reconstitution. **Reconstituted standards and controls can be stored at -20 °C.** Avoid repeated freeze/thaw cycles.

## 7. PRECAUTIONS

- To avoid cross-contamination, change pipette tips between addition of standards, controls, samples, antibody, conjugate and substrate. Also use separate reservoirs for each reagent.
- Avoid foaming when mixing the reagents.
- Do not mix stoppers and caps of different reagents.
- Do not use reagents beyond expiration date.
- Do not mix or substitute reagents with those from other lots or sources.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate solution should remain colourless until added to the plate.
- Stop solution should be added to the plate in the same order as the substrate solution.
- Protect reagents from direct sunlight.
- Do not pipette by mouth.
- Do not eat, drink, smoke or apply cosmetics where reagents are used.
- Avoid all contact with the reagents by using gloves.

- Stop solution is composed of sulfuric acid, which is a strong acid. Even diluted, it still must be handled with care. It can cause acid burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spills should be wiped out immediately with copious quantities of water.
- Human materials used in kit components were tested and found to be negative for HIV, Hepatitis B and Hepatitis C. However, for safety reasons, all kit components should be treated as potentially infectious.

## 8. SPECIMEN COLLECTION AND PREPARATION

### *Serum samples*

Serum samples can be used without further dilution. Samples have to be stored at  $-20\text{ }^{\circ}\text{C}$  if not measured the same day.

### *Measurement of sRANKL in Cell Culture Supernatants*

The stock solution have to be reconstituted with **2 ml** cell culture medium. Allow the vial to stand for 10 minutes and then mix thoroughly by gentle conversion to insure complete reconstitution This stock solution contains an sRANKL (**20 pmol/l**). Prepare a serial dilution of the sRANKL stock solution with cell culture medium, e.g. **20/10/5/2.5/1.25 pmol/l**. The choice of the standard range is dependent on the amount of sRANKL secreted by the respective cell type. Cell culture medium is used as a 0-standard. If necessary, dilute cell culture supernatant according to the expected concentration with cell culture medium. Dilution of the supernatant depends on the sRANKL amount secreted by the respective cell type.

## 9. ASSAY PROCEDURE

### *Procedural notes*

- Do not interchange different lot numbers of any kit component within the same assay.
- The quality control guidelines should be followed.
- Incubation time, incubation temperature and pipetting volumes of the different components are defined by the manufacturer. Any variations of the test procedure, that are not coordinated with the producer, may influence the test results. Immundiagnostik can therefore not be held responsible for any damage.
- The assay should always be performed according the enclosed manual.

### *Test procedure*

It is recommended to perform the assay in duplicate and to cover the microplate wells during the incubation steps.

1. Pipette **100µl** of standards, control or samples in the respective wells.
2. Add **100µl** detection antibody (glass vial, green solution) to all wells, except blank. Mix gently.
3. Cover strips with plastic film and incubate overnight (16 - 24 hours) at 2-8°C. To avoid evaporation, make sure that all wells are sealed well with the plastic film.
4. Remove plastic film carefully and discard contents of the wells. Wash **5x** with minimum **300 µl** diluted wash buffer. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper to remove excess solution.
5. Add **200 µl** conjugate (red solution) to all wells and cover strips with plastic film.
6. Incubate for 60 min. at room temperature (18-26°C).
7. Remove plastic film carefully and discard contents of the wells. Wash **5x** with minimum **300 µl** diluted wash buffer. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper to remove excess solution.
8. Add **200µl** of substrate solution to all wells and incubate for 30 min. at room temperature (18-26°C) in the dark.
9. Add **50 µl** stop solution to all wells, mix gently.
10. Determine absorption immediately with an ELISA reader at 450 nm against 620 nm (or 690 nm) as a reference. If no reference wavelength is available, read only at 450 nm.
11. If the extinction of the highest standard exceeds the range of the photometer, absorption must be measured immediately at 405 nm against 620 nm as a reference.

## 10 RESULTS

The extinction of the blank is subtracted from all other values. A calibration curve is constructed from the standards. Commercially available software can be used as well as graph paper. Results of the samples are read from this calibration curve.

The assay has been evaluated using a 4 PL algorithm. Different curve fitting methods need to be evaluated by the user.

Respective dilution factors have to be considered.

## 11. LIMITATIONS

Samples with sRANKL levels greater than the highest standard value should be further diluted and re-assayed.

## 12. QUALITY CONTROL

Immundiagnostik AG recommends the use of commercial control samples for internal quality control if available.

Control samples should be analyzed with each run. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results for the patient samples may not be valid, if within the same assay one or more values of the quality control sample are outside the acceptable limits.

### *Expected values*

**Conversion factor for pg/ml to pmol/l**

1pg/ml=0.05pmol/l; Mol. mass of recombinant sRANKL standard - 20 kD

## 13. PERFORMANCE CHARACTERISTICS

### *Precision and reproducibility*

#### **Intra-Assay (Precision within an assay)**

2 samples of known concentrations were tested 16 times to assess intra-assay precision:

Intra-Assay Precision n=16	#1	#2
Mean (pmol/l)	1.0	3.2
<b>SD</b>	0.04	0.09
<b>CV %</b>	5	3

## Inter-Assay (Precision between assay)

Two samples of known concentrations were tested in 10 assays to assess inter-assay precision:

Inter-Assay Precision n=10	#1	#2
Mean (pmol/l)	0.8	1.78
SD	0.07	0.11
CV %	9	6

## Sensitivity

The detection limit (0 pg/ml + 3 SD): 0.08 pmol/l

## 14. REFERENCES

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- 12) Hofbauer L.C. et al., Effects of oral contraceptives on circulating osteoprotegerin and soluble RANK ligand serum levels in healthy young women. (2004) *Clin Endocrinol* 60:214-219

## 15. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- This assay was produced and put on the market according to the IVD guidelines of 98/79/EC.
- The test components which are made of human serum are tested for Australia antigen and HIV and found to be negative. However, since no test method can offer complete assurance that infectious agents are absent, these reagents should be handled as recommended for any potentially infectious human serum or blood specimen. The normal precautions for laboratory working should be observed.
- Kit reagents contain sodium azide or thimerosal as bactericides. Sodium azide and thimerosal are toxic. Substrates for the enzymatic color reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes.
- For *in vitro* diagnostic use only.
- Reagents should not be used beyond the expiration date shown on the kit label.
- Do not mix different lot numbers of any kit component.
- Quality control guidelines should be followed.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. Immundiagnostik AG can therefore not be held responsible for any damage resulting from wrong use.
- Warranty claims and complaints in respect of deficiencies must be logged within 14 days after receipt of the product. The product should be send to Immundiagnostik AG along with a written complaint.

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Li StarFish distribuisce:

